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The Antimicrobial Activity of Essential Oils of Al-Abbas′s (AS) Hand Fruit Peel *(*Citrus Medica) var. Sarcodactylis Swingle

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Abstract
A study was conducted to determine the effect of essential oils of Al-Abbas′s (AS) Hand fruit Peel (*Citrus medica) var. sarcodactylis Swingle* on the Pathogenic bacteria. Al-Abbas′s (AS) Hand fruit peels essential oils extracted by hydro-distillation using a Clevenger-type apparatus, the oil yield was about 0.75% (7.5 g/1 kg) of fresh peel. The essential oils of Al-Abbas′s (AS) Hand (*Citrus medica) L. var. sarcodactylis (Sieber) Swingle* fruit peel has effective antibacterial activities on the test isolates as indicated by the diameter of their zone of inhibition. The inhibition zone was 18 mm for *Enterobacter cloacae*, 15 mm for *Escherichia coli* and *Pseudomonas aeruginosa*, 22 mm for *Klebsiella pneumoniae*, 20 mm for *Proteus mirabilis*, *Bacillus sp.* and *Streptococcus spp.,* 25 mm for *Staphylococcus aureus*. The study revealed that the essential oils of Al-Abbas′s (AS) Hand fruit peel could be as a therapeutic agent for human microbial infections.
Keywords: Essential oils, *Citrus medica var. sarcodactylis Swingle*, peel extract, pathogenic bacteria antimicrobial activity.

1. Introduction

The finger citron (*Citrus medica* var. sarcodactylis Swingle) (FC) is a medicinal plant from the Rutaceae family. Customarily, it is more popularly named “Buddha hand citron”, “Longevity orange”, or “Five finger orange” in commercial vegetable markets [1]. This plant is of economic importance since it has been used as a traditional Chinese medicinal material and functional vegetables, and preserved as sweetmeats [2, 3]. In Oriental countries, the finger citron fruits are extensively consumed as functional vegetables in a specific diet. As a medicinal plant, the finger citron has been credited with a long list of medicinal uses. Because traditionally finger citron fruits have been considered to be beneficial to pancreas, liver and stomach functions, and people like to take finger citron fruits as adjuvant herbal medicines to treat a diversity of chronic diseases like asthma, hypertension and respiratory tract infections [2, 3, 4]. It is worth noting that the bioactive substances contained in the finger citron plays an important role in its biological activity. Moreover, diets rich in selected natural antioxidants such as the essential oils (EOs) and various extracts from finger citron are related to reduced risk of incidence of hyperlipidemia, obese and other chronic diseases has lead to the revival of interest in plants-based foods [3, 4].

Chemical composition of EOs obtained from the finger citron fruits was greatly influenced by the maturation stages as mentioned by Peng *et al*. (2009). Additionally, variations of the EOs composition in many different fruits have been observed, depending on genetic and environmental factors as well as ontogeny and analytical methods [5, 6, 7, 8, 9, 10, 11]. Maturation stages constitute an important factor influencing chemical composition and biological activities of the EOs extracted from fruits, vegetables and medicinal plants [11, 12, 13, 14, 15]. The ability to determine suitable harvesting periods would increase the grower’s control on both fruit yield and essential oils quality in the FC fruits due to shattering possibility of fully mature fruits. Delayed harvests can result in fruits on the primary fruit shattering, browning and decay. Early harvesting also causes yield losses due to premature fruits. Despite records on EOs composition of FC fruits [2, 16], to the best of our knowledge, chemical composition and antioxidant activities variation of EOs extracted from FC fruits in different maturation stages have not yet been reported. The present work was undertaken with the main objective to investigate the chemical composition of the EOs isolated from FC fruits as affected by three different maturation stages along with their antioxidant activities.

Essential oils are natural products that plants produce for their own needs other than nutrition such as protection or attraction. In general, they are complex mixtures of organic compounds that give characteristic odour and flavour to the plants. They are mainly made up by monoterpenes and sesquiterpenes whose main metabolic pathway is through mevalonate leading to sesquiterpenes and from methyl-erythritol leading to monoterpenes.

They are located in different parts of the plant. They can be found in the root such as that of the vetiver grass, in stems like that of peteribi wood and incense, in leaves like in eucalyptus trees, citronella, chinchilla and lemon grass, in flowers like lavenders, in fruit like lemon, orange and even in seeds as in the case of anise, coriander and pepper, among others [17]. They can work as internal messengers, like defense substances or plant volatiles aimed at natural enemies but also to attract pollinating insects to their host [18].

Essentials oils are accumulated in cells, secretory cavities or glandular hairs of plants. They are globules with impermeable cells (stomata) whose interior have essentials oils. In the case of citrus, stomata can be

observed at first sight because they are macroscopic. Apart from superior plants, some land and sea animals, insects, mushrooms and microorganisms are also known for the biosynthesis of similar volatile compounds [19].

In general, essential oils have a nice smell, that is why they are used in different industries, especially in perfumes (fragancias and lotions), in foodstuff (like flavoring and preservatives) and in pharmaceutical products (therapeutic action) [20].

There are different methods for essential oil extraction. One of the most common is steam distillation since it allows for the separation of slightly volatile, water-inmiscible substances by means of low temperature distillation, being of particular use when the components boil at high temperature (higher than 100ºC) and are susceptible to decomposition below this temperature [17].

In order to get oil from citrus fruits, such as oranges, lemons or tangerines, cold expression is preferred, due to the thermal instability of the main constituents of the essential oil. The oil cells are located below the epicarp surface. The fruit must be washed and sliced into two halves, the pulp withdrawn and then the peel must be softly pressed to break the oil glands, which can be removed with water.

From the chemical point of view, the essential oil composition frequently changes in different parts of the plant. Quite often, between the different organs of the plant, phytochemical polymorphism can be produced. As an example, in Origanum vulgare ssp. hirtum, polymorphism could be detected, even within one individual plant, between different oil glands of a single leaf [21]. However, this kind of polymorphism is not very usual, being the difference in the oil composition between glands usually related to gland age [21, 22, 23, 24]. In general, the differe-nt growth stages of the plant create variations in the oil composition within the same organ of the plant [24].

Essential oils possess a wide spectrum of different impressive qualities including antiphlogistic, spasmolythic, antinociceptive and antioxidant activity. Moreover they exert immunomodulant, psychotrope, acaricide and expectorant effects [25]. Due to their multifunctionality, Essential oils fined a huge application area in medicine and aromatherapy. Also antiviral, antidiabetic and cancer suppressive activities are observed. In addition to further other effects, Essential oils show significant antimicrobial properties against a wide range of Gram-positive and Gram-negative bacteria. That is why they were already used for embalming in Ancient Egypt [26].

In the course of history Essential oils were always applied for their antimicrobial effects in traditional medicine. Therefore, plants were used for the treatment of infectious illnesses since ancient times even though no knowledge about microorganisms existed by then [27].

In Iraq the (Citrus medica) var. sarcodactylis Swingle called Al-Abbas's Hand fruit ,the raw fruit peel and extracts are highly regarded in Iraq for their many medicinal uses (ranging from Alzheimer's to cancer to diabetes to ulcers to intestinal parasites) and as an insect repellent. People in Iraq believed that cultivation of this plant brings them luck and livelihood. In the present study essential oils extracted from (Citrus medica) var. sarcodactylis Swingle fruits peel and evaluation of their antibacterial activity against various human multi drug resistant pathogenic bacteria.

2. Methods and materials

2.1 Collection of pathogens

We are collected multiple antibiotic-resistant isolates, (Gram positive, and Gram negative) which included *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Streptococcus sp*., *Enterobacter cloacae*, *Bacillus sp*., *Pseudomonas aeruginosa*, and *Staphylococcus aureus* used for the antimicrobial activity from microbiology diagnosis laboratory, Al-Numan hospital.

2.2 Plants collection

Fresh Al-Abbas's (AS) hand fruit (*Citrus medica) var. sarcodactylis Swingle* used in this study were obtained from the local market at Zakho City, Iraq. The fresh fruits were washed in running tap water in laboratory, surface sterilized with 70% alcohol, rinsed with sterile distilled water. The fruits were then peeled off carefully with the help of a sharp knife to avoid any damage of oil glands.

2.3 Extraction of Al-Abbas's hand fruit Essential Oil

One kilo grams of the Al-Abbas's (AS) hand fruit peels were chopped in a blender; chopped peels were subjected to hydrodistillation using a *Clevenger apparatus of the quality control laboratory of the General Company for Vegetable Oil Industry, Baghdad,* for 5 h for isolation of oils [28]. Mixture of (*Citrus medica) L. var. sarcodactylis (Sieber) Swingle* oils and water was incorporated which was separated, in two liquid layers which was isolated. Distillates of essential oils were dried over anhydrous sodium sulfate, filtered and then concentrated at 30°C using a rotary evaporator (Buchi R-124, Flavil, Sweden) and the resulting yellowish EOs was subsequently stored in dark brown sealed vials at -20°C until use.

2.4 Antimicrobial activities

The screening of antimicrobial activities of essential oils extract on the tested bacteria used in this investigation was determined on Muller Hinton agar media (all tested organism grow on Muller Hinton agar media), by the using agar well diffusion method. Wells of 6 mm diameter and 5 mm depth were made on the solid agar using a sterile cork borer [29]. 100µl of essential oils extract was inoculated onto wells were made in the spread plate culture of each microbial isolates (each microbial concentration was made 10^6 CFU/ml). The plates were performed in triplicates. All plate of the tested organisms was then allowed to incubate at 37°C for overnight. After 24 h of incubation. The diameters of the zone of inhibitions were measured by measuring scale in millimeter (mm).

3. Results

The Al-Abbass (AS) Hand fruit (*Citrus medica*) is one of the three primordial citrus fruit (the others being pummelo and mandarine) from which most other citrus originated. It is an evergreen tree, ranging in height from 3 to 5 m, with fruit borne on thorny branches in 2-3 waves during the year. The tree is relatively short-lived (up to 15-18 years) and is sensitive to many insects and soil diseases, as well as to high and low temperatures (figure 1). Fruit range in size from 200 to 800 g, although they can grow much larger. Fruit attain their size while the peel is still green, and then ripen to yellow or even orange (figure 2). The oil yield was about 0.75% (4.55 g/500 g of fresh peel.

Figure (1) showed the tree of Al-Abbas′s (AS) Hand fruit (*Citrus medica) var. sarcodactylis Swingle.*

Figure (2) showed the shape Al-Abbas′s (AS) Hand fruit (*Citrus medica) var. sarcodactylis Swingle*.

The data in Table (1) and Figure (3, 4, 5) shows that essential oils of Al-Abbas′s (AS) Hand fruit (*Citrus medica) var. sarcodactylis Swingle* fruit peel has effective antibacterial activities on the test isolates as indicated by the diameter of their zone of inhibition. The inhibition zone was 18 mm for *Enterobacter cloacae*, 15 mm for *Escherichia coli* and *Pseudomonas aeruginosa*, 22 mm for *Klebsiella pneumoniae*, 20 mm for *Proteus mirabilis*, *Bacillus sp.* and *Streptococcus spp.,* 25 mm for *Staphylococcus aureus*.

Table (1). The inhibitory activity of the essential oils extracted from Al-Abbas′s (AS) Hand fruit (*Citrus medica) var. sarcodactylis Swingle* fruit peel against the tested bacteria as demonstrated by diameters of the inhibition zone (mm)*.

	Zone of Inhibition
Isolated bacteria	100µl essential oils of Al-Abbas′s (AS) Hand fruit peel
Enterobacter cloacae	18
Escherichia coli	15
Klebsiella pneumoniae	22
Proteus mirabilis	20
Pseudomonas aeruginosa	15
Bacillus sp.	20
Staphylococcus aureus	25
Streptococcus spp.	20

* Zone of inhibition, including the diameter of the cup plate method (8.0 mm) .The recorded value is mean value of 3 replicates.

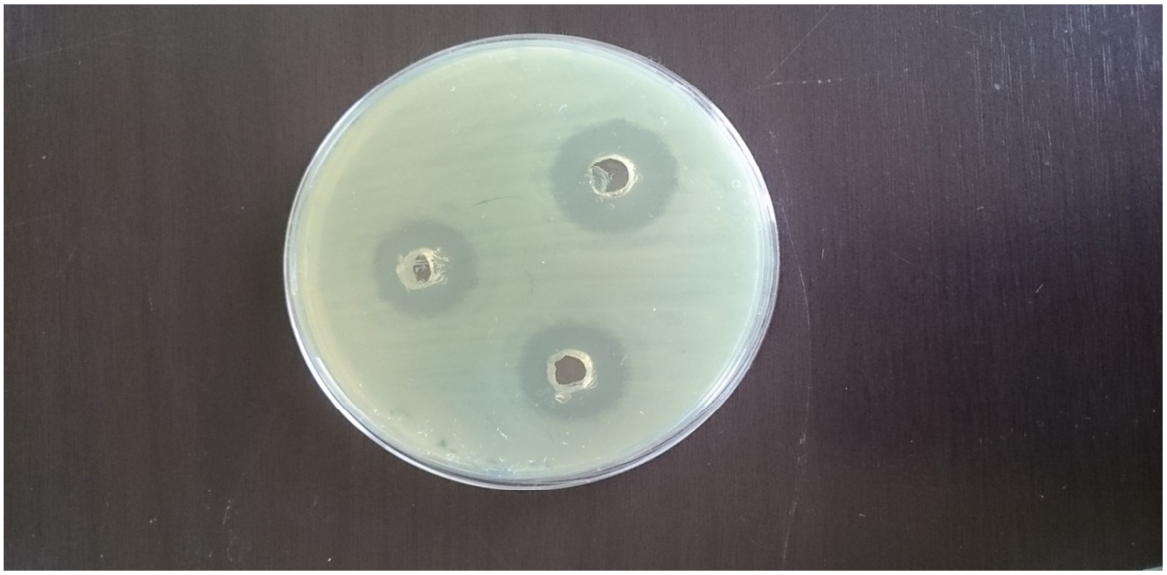

Figure (3). The antibacterial effect of essential oils of Al-Abbas′s (AS) Hand fruit (*Citrus medica) var. sarcodactylis Swingle* fruit peel against *Escherichia coli*.

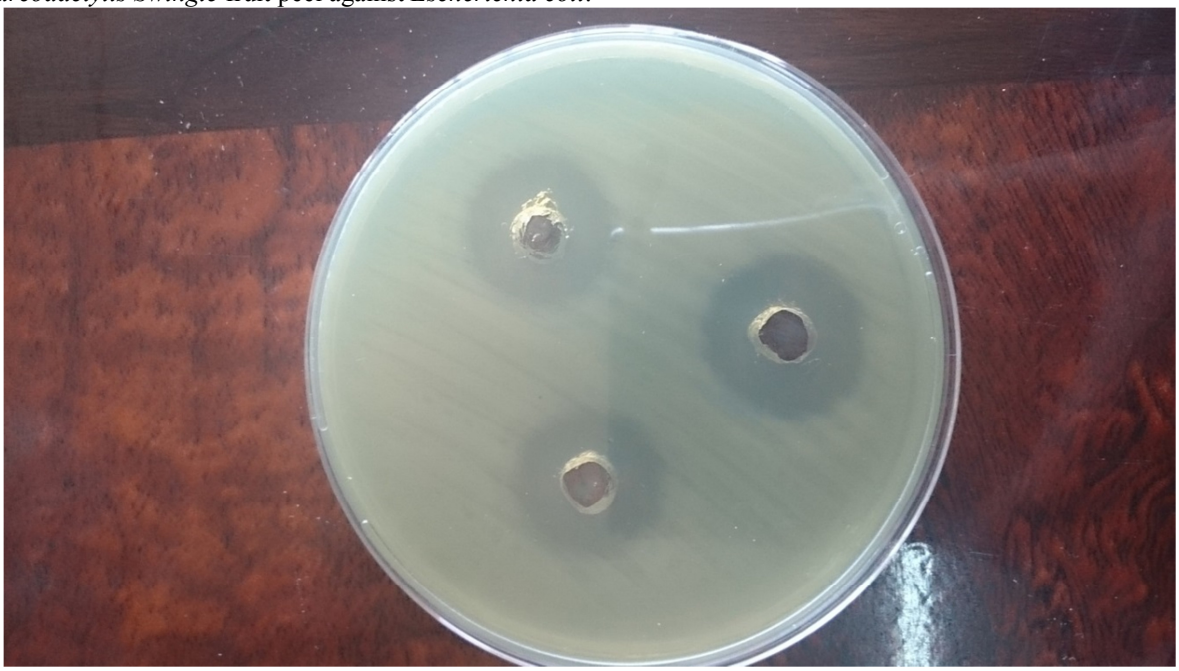

Figure (4). The antibacterial effect of essential oils of Al-Abbas′s (AS) Hand fruit (*Citrus medica) var. sarcodactylis Swingle* fruit peel against *Klebsiella pneumoniae*.

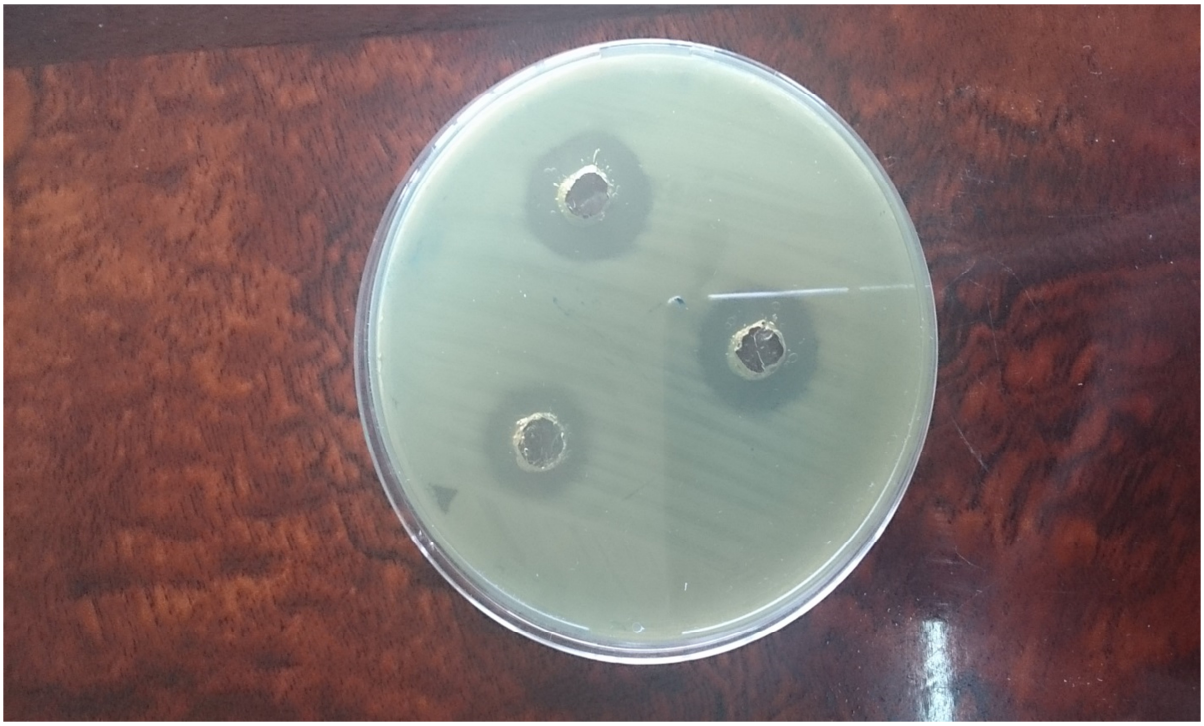

Figure (5). The antibacterial effect of essential oils of Al-Abbas′s (AS) Hand fruit (*Citrus medica) var. sarcodactylis Swingle* fruit peel against *Staphylococcus aureus*.

4. Discussion

Citrus medica L. var. sarcodactylis belonging to the family Rutaceae has originated in India and has been worldwide spread to other regions following the paths of civilization [30]. It is known as fingered citron or as fo-shou (Buddha's hand) in China [31]. In Iraq this type of trinj is rare and it is known in Diyala city and some parts of north of Iraq as Al-Abbas's Hand fruit and used in folk medicine as tonic, antispasmodic, antiemetic, expectorant and inhaler. People in Iraq believe that cultivation of this plant brings them luck and livelihood. Recent studies reported that *C. medica* L. var . sarcodactylis constituted coumarin compounds, p-coumaric acids, steroids, triterpenoids, limonin, nomilin, etc [32, 33].

The major volatile components in the peel oil from the Japanese fingered citron were limonene and γ-terpinene [16]. According to scientific literatures, no scientific information on the chemical composition and bioactivity for essential oil from *C. medica* L. var. sarcodactylis leaf has been reported.

In the present study the method used to isolate the essential oils from Al-Abbas′s (AS) Hand fruit (*Citrus medica*) L. var. sarcodactylis (Sieber) Swingle peel *was hydro- distillation by using the clevenger apparatus of the quality control laboratory of the General Company for Vegetable Oil Industry, Baghdad.*

The percentage of oils isolation was acceptable compared to many research that extracting oil from different parts of Citrus spp The result of our study are agreement with several previous studies in which the isolation ratio ranged from 0.27% to 1.5% [34, 35, 36, 37].

The results of present study shows that essential oils of Al-Abbas′s (AS) Hand fruit (*Citrus medica*) L. var. sarcodactylis (Sieber) Swingle peel has effective antibacterial activities on the test isolates as indicated by the diameter of their zone of inhibition, and the effects on Gram- positive bacteria was more than the effect on Gram- negative bacteria, these finding agreement with former studies indicate a higher antibacterial effect of essential oils against Gram-positive than against Gram-negative bacteria. The outer cell membrane of Gram-negative bacteria obtains hydrophilic qualities that impede the contact of the hydrophobic constituents of the essential oil with the bacterial cell [38, 39].

Contrary to this, essential oils can directly impair the cell membrane of Gram-positive bacteria leading to cell membrane rupture, blocking of enzyme systems and progressivity of ion permeability [40, 41].

The increasing tolerance of several microorganisms against commonly used antibiotic drugs represents a challenge for scientists to find alternative ways for the treatment of such infections. One of the main causes that provokes the higher resistance of microorganisms is the loose application of drugs [42]. This includes that they are applied in too low concentrations, not specific enough or without serious indication. Especially methicillin-resistant *S. aureus* (*MRSA*) strains are popular test microorganisms *S. aureus* – a Gram-positive bacterium which is common part of the human microbial skin flora - can cause minor infections, but nevertheless also severe diseases such as pneumonia, sepsis, endocarditis or meningitis particularly in hospitalized patients.

The increasing resistance of these pathogens against current drugs tremendously complicates the therapy of these infections [42, 43].

The mechanism of essential oils to killing Gram-negative bacteria belong to the essential oils which contained asteraceae among other constituents geraniol showed an inhibitory activity against multidrug resistant strains of the Gram-negative bacteria *Escherichia coli*, *Klebsiella oxytoca*, *Proteus mirabilis* and *P. aeruginosa*. This finding agreement with the result of two studies [35, 44].

Our results support the hypothesis that essential oils can be prepared in a simple and cost-effective manner and are suitable for formulation of new types of bacteri-cidal materials.

5. Conclusions

The broad spectrum of antimicrobial activities of the essential oils against variety of bacterial strains so we can conclude that the essential oil of Al-Abbas's hand fruit peel can be incorporated in different pharmaceutical preparations.

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الفعالية المضادة للجراثيم للزيوت الاساسية المستخلصة من قشور فاكهة كف العباس (ع.س.) سترس مديكا ل. فار. ساركوداكتيليس (سيبر) سونجل.

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قسم علوم الحياة ، كلية التربية للعلوم الصرفة ابن الهيثم، جامعة بغداد.

الخلاصة
أجريت الدراسة لتحديد تأثيرالزيوت الأساسية لقشور فاكهة كف العباس (ع.س.) سترس مديكا ل. فار. ساركوداكتيليس (سيبر) سونجل على البكتيريا المسببة للأمراض. استخلصت الزيوت الأساسية من قشور فاكهة كف العباس (ع.س.) بطريقة التقطير المائي باستخدام جهاز كليفنجر، نسبة الزيوت االمنتجة كانت 0.75% (7.5غم/1كغم) من القشور الطازجة. ثبطت الزيوت الأساسية المستخلصة من قشور فاكهة كف العباس (ع.س.) سترس مديكا ل. فار. ساركوداكتيليس (سيبر) سونجل الجراثيم المسببة للأمراض المختبرة واستدل على دلك من قطر منطقة التثبيط. ملم للأمعائية المذرقية، 15 ملم للاشريكية القولونية **والزائفة الزنجارية، 22 ملم للكليبسيلا الرئوية، 20 ملم للمتقلبة منطقة التثبيط كانت 18 الرائعة، العصوية س. و العقدية س.، 25 ملم للمكورات العنقودية الذهبي. كشفت الدراسة أن الزيوت الاساسية المعزولة من قشور فاكهة كف العباس (ع.س.) يمكن أن تستخدم كعامل علاجي للعدوى الميكروبية للانسان.**

الكلمات المفتاحية: الزيوت الاساسية، فاكهة كف العباس (ع.س.)، سترس مديكا ل. فار. ساركوداكتيليس (سيبر) سونجل ، الجراثيم المرضية، الفعالية المضادة للجراثيم.

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